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TITLE

OB PROTEIN RECEPTOR AND RELATED COMPOSITIONS AND METHODS

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EXHIBIT 3
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OB PROTEIN RECEPTOR AND RELATED COMPOSITIONS AND METHODS

FIELD OF THE INVENTION

The present invention relates to OB protein receptors, related compositions and methods of making and using such receptor and related compositions.

BACKGROUND

Although the molecular basis for obesity is largely unknown, the identification of the "OB gene" and protein encoded ("OB protein") has shed some light on mechanisms the body uses to regulate body fat deposition. Zhang et al., Nature 372: 425-432 (1994); see also, the Correction at Nature 374: 479 (1995). The OB protein is active in vivo in both ob/ob mutant mice (mice obese due to a defect in the production of the OB gene product) as well as in normal, wild type mice. The biological activity manifests itself in, among other things, weight loss. See generally, Barinaga, "Obese" Protein Slims Mice, Science 269: 475-476 (1995).

The other biological effects of OB protein are not well characterized. It is known, for instance, that in ob/ob mutant mice, administration of OB protein results in a decrease in serum insulin levels, and serum glucose levels. It is also known that administration of OB protein results in a decrease in body fat. This was observed in both ob/ob mutant mice, as well as non-obese normal mice. Pelleymounter et al., Science 269: 540-543 (1995); Halaas et al., Science 269: 543-546 (1995). See also, Campfield et al., Science 269: 546-549 (1995)(Peripheral and central administration of microgram doses of OB protein reduced food intake and body weight of *ob/ob* and diet-induced obese mice but not in *db/db* obese mice.) In none of these reports have toxicities been observed, even at the highest doses.

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Despite the promise of clinical application of the OB protein, the mode of action of the OB protein *in vivo* is not clearly elucidated, in part due to the absence of information on the OB receptor. High affinity binding of the OB protein has been detected in the rat hypothalamus, reportedly indicating OB receptor location. Stephens et al., Nature 377: 530-532. The *db/db* mouse displays the identical phenotype as the *ob/ob* mouse, *i.e.*, extreme obesity and Type II diabetes; this phenotype is thought to be due to a defective OB receptor, particularly since *db/db* mice fail to respond to OB protein administration. *See* Stephens et al., *supra*.

Identification of the OB protein receptor is key in determining the pathway of signal transduction. Moreover, identification of the OB protein receptor would provide powerful application in diagnostic uses, for example, to determine if individuals would benefit from OB protein therapy. Furthermore, the OB receptor could be a key component in an assay for determining additional molecules which bind to the receptor and result in desired biological activity.

SUMMARY OF THE INVENTION

The present invention relates to a novel class of protein receptors, herein denominated "OB protein receptors" or "OB receptors", which are thought to selectively bind OB protein. As such, the novel OB receptor family is provided, as well as novel members of such family. Also provided are nucleic acids, vectors and host cells containing such nucleic acids, related antisense nucleic acids, molecules which selectively bind to the OB protein receptor, and related compositions of matter. In other aspects, the present invention relates to methods of using the above compositions, such as diagnostic methods, and methods for preparing OB receptor ligands.

DETAILED DESCRIPTION

A novel family of OB receptors is provided. This novel family resulted from identification of a PCR fragment isolated from a human liver cell cDNA library. The original PCR fragment, from which primers were isolated, contained a "WSXWS" motif, common to cytokine receptors. As illustrated by the working examples below, using this fragment three members of this OB protein receptor family have been identified. These members, herein designated as "A", "B", and "C", are indentical at amino acid position 1-891 (using the numbering of Seq. ID No. 1), but diverge at position 892 through the C terminus. They vary in length at the C-terminus beyond amino acid 891, and the different forms appear to have different tissue distribution.

Ligand binding may be localized to the extracellular domain of the OB receptor. Using hydrophobicity analysis, the leader sequence is likely to comprise amino acids (Seq. ID. No. 1) 1-21, 1-22, or 1-28. The first amino acid of the mature protein is likely to be 22(F), 23(N) or 29(T). Most likely, the first amino acid of the mature protein is 22 (F). The beginning of the transmembrane domain appears to be located at position 839(A) or 841(L). The end of the transmembrane domain appears to be located at position 862(I), 863(S) or 864(H). Thus, based on predictions from hydrophobicity analysis, for OB protein binding, at a minimum what is needed is the extracellular domain of the mature protein, amino acids 22, 23 or 29 through amino acids 839(D) or 841(G). Therefore, the present class of OB receptor proteins includes those having amino acids (according to Seq. ID No. 1):

(a) 1-896;
(b) 22-896;
(c) 23-896;
(d) 29-896

(e) 1-839;
(f) 22-839;
(g) 29-839;
(h) 1-841;
(i) 22-841;
(j) 23-841;
(k) 29-841;
(l) 1-891;
(m) 22-891;
(n) 23-891;
(o) 29-891;
(p) the amino acids of subparts (l) through (o) having the C-terminal amino acids of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5);
(q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

The C-terminus region is intracellular. The differences in the C-terminus among members of the present OB recepter family may result in differences in signal transduction among the species. Thus, the present OB receptors include at least the extracellular domain which is important for OB protein ligand binding. Nucleic acids encoding the present OB receptors, vectors, and host cells are also provided for herein.

Human genomic DNA is also provided herein. The genomic DNA has been localized to human chromosome 1P31, which is believed to correspond to mouse chromosome 4, the presumed location of the mouse *db* locus.

Tissue distribution analysis demonstrates the presence of OB receptor nucleic acids is fairly ubiquitous, and particularly noted in the liver. It is also observed in the ovary, and heart; and, to a lesser extent, in small intestine, lung, skeletal muscle, kidney, and, to an even

lesser extent, spleen, thymus, prostate, testes, placenta and pancreas.

Nucleic Acids

According to the present invention, novel OB protein receptors and DNA sequences coding for all or part of such OB receptors are provided. Novel nucleic acid sequences of the invention include sequences useful in securing expression in procaryotic or eucaryotic host cells of polypeptide products having at least a part of the primary structural conformation and one or more of the biological properties of recombinant human OB receptor. The nucleic acids may be purified and isolated, so that the desired coding region is useful to produce the present polypeptides, for example, or for diagnostic purposes, as described more fully below. DNA sequences of the invention specifically comprise: (a) any of the DNA sequence set forth in Seq. ID No.2, 4, 6 and 7 (and complementary strands); (b) a DNA sequence which hybridizes (under hybridization conditions disclosed in the cDNA library screening section below, or equivalent conditions or more stringent conditions) to the DNA sequence in subpart (a) or to fragments thereof; and (c) a DNA sequence which, but for the degeneracy of the genetic code, would hybridize to the DNA sequence in subpart (a). Specifically comprehended in parts (b) and (c) are genomic DNA sequences encoding allelic variant forms of human OB receptor and/or encoding OB receptor from other mammalian species, and manufactured DNA sequences encoding OB receptor, fragments of OB receptor, and analogs of OB receptor which DNA sequences may incorporate codons facilitating transcription and translation of messenger RNA in microbial hosts. Such manufactured sequences may readily be constructed according to the methods of Alton et al., PCT published application WO 83/04053.

Genomic DNA encoding the present OB receptors may contain additional non-coding bases, or introns, and such genomic DNAs are obtainable by hybridizing all or part of the cDNA, illustrated in Seq. ID NOs. 1, 3, and 5, to a genomic DNA source, such as a human genomic DNA library. Such genomic DNA will encode functional OB receptor polypeptide; however, use of the cDNAs may be more practicable in that, since only the coding region is involved, recombinant manipulation is facilitated.

Such sequences include the incorporation of codons "preferred" for expression by selected nonmammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences which facilitate construction of readily expressed vectors. The present invention also provides DNA sequences coding for polypeptide analogs or derivatives of OB receptor which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (i.e., deletion analogs containing less than all of the residues specified for OB receptor; substitution analogs, wherein one or more residues specified are replaced by other residues; and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the polypeptide) and which share some or all the biological properties of OB receptor.

Such substitutions may include the substitution of serine or alanine at one or more of the cysteinyl residues, conserved amino acid substitutions (conserved in terms of charge, hydrophobicity or both) or other substitutions. The leader sequence DNA may be substituted with another leader sequence for ease in expression or for other purposes.

The present DNA sequences may be selected from among those encoding OB receptors including those having amino acids (according to Seq. ID No. 1):

(a) 1-896;
(b) 22-896;
(c) 23-896;
(d) 29-896
(e) 1-839;
(f) 22-839;
(g) 29-839;
(h) 1-841;
(i) 22-841;
(j) 23-841;
(k) 29-841;
(l) 1-891;
(m) 22-891;
(n) 23-891;
(o) 29-891;
(p) the amino acids of subparts (l) through (o) having the C-terminal amino acids of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5);and
(q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

The present invention also includes human genomic DNA.

In addition, since the C-terminus region of the above polyeptides diverges at position 892 (with respect to Seq. ID Nos. 1, 3, and 5) one may desire to prepare only that portion of the polypeptides which are divergent. As such, DNA sequences are provided which encode polypeptides:
(a) having only amino acids 892-896 of Seq. ID No. 1;
(b) having only amino acids 892-933 of Seq. ID No. 3;
(c) having only amino acids 892-959 of Seq. ID No. 5.

Also, one may prepare antisense nucleic acids against the present DNAs. Such antisense nucleic acids may be useful in modulating the effects of OB protein _in vivo_. For example, one may prepare an antisense nucleic acid which

effectively disables the ability of a cell to produce OB receptor by binding to the nucleic acid which encodes such OB receptor.

DNA sequences of the invention are also suitable materials for use as labeled probes in isolating human genomic DNA encoding OB receptor, as mentioned above, and related proteins as well as cDNA and genomic DNA sequences of other mammalian species. DNA sequences may also be useful in various alternative methods of protein synthesis (e.g., in insect cells) or, as described above, in genetic therapy in humans and other mammals. DNA sequences of the invention are expected to be useful in developing transgenic mammalian species which may serve as eucaryotic "hosts" for production of OB receptor and OB receptor products in quantity. See, generally, Palmiter et al., Science 222: 809-814 (1983).

Vectors and Host Cells

According to another aspect of the present invention, the DNA sequences described herein which encode OB receptor polypeptides are valuable for the information which they provide concerning the amino acid sequence of the mammalian protein which have heretofore been unavailable. Put another way, DNA sequences provided by the invention are useful in generating new and useful viral and circular plasmid DNA vectors, new and useful transformed and transfected procaryotic and eucaryotic host cells (including bacterial and yeast cells and mammalian cells grown in culture), and new and useful methods for cultured growth of such host cells capable of expression of OB receptor and its related products.

The DNA provided herein (or corresponding RNAs) may also be used for gene therapy for, example, treatment of conditions characterized by the overexpression of OB protein, such as anorexia or cachexia. Alternatively, gene therapy may be used in cases where increased sensitivity to OB protein is

desired, such as in cases where an individual has a condition characterized by OB protein receptors defective in ability to bind or retain the binding of OB protein. Currently, vectors suitable for gene therapy (such as retroviral or adenoviral vectors modified for gene therapy purposes and of purity and pharmaceutical acceptability) may be administered for delivery into the lung, for example. Such vectors may incorporate nucleic acid encoding the present polypeptides for expression in a desired location. Gene therapy may involve a vector containing more than one gene for a desired protein.

Alternatively, one may use no vector so as to facilitate relatively stable presence in the host. For example, homologous recombination may facilitate integration into a host genome. (This may be performed for production purposes as well, e.g., U.S. Patent No. 5,272,071 and WO 91/09955.) The nucleic acid may be placed within a pharmaceutically acceptable carrier to facilitate cellular uptake, such as a lipid solution carrier (e.g., a charged lipid), a liposome, or polypeptide carrier (e.g., polylysine). A review article on gene therapy is Verma, Scientific American, November 1990, pages 68-84 which is herein incorporated by reference.

As mentioned above, target cells may be within the lungs of the recipient, but other target cells may be adipocytes or precursors thereof, bone marrow cells, blood cells, such as peripheral blood progenitor cells, liver (or other organ) cells, muscle cells, fibroblasts, or other cells. The desired nucleic acid may be first placed within a cell, and the cell may be administered to a patient (such as a transplanted tissue) or the desired nucleic acid may be administered directly to the patient for uptake <u>in vivo</u>.

The cells to be transferred to the recipient may be cultured using one or more factors affecting the growth or

proliferation of such cells, as for example, SCF if appropriate..

For gene therapy dosages, one will generally use between one copy and several thousand copies of the present nucleic acid per cell, depending on the vector, the expression system, the age, weight and condition of the recipient and other factors which will be apparent to those skilled in the art.

Thus, the present invention provides for a population of cells expressing an OB receptor of the present OB receptor family. Such cells are suitable for transplantation or implantation into an individual for therapeutic purposes. For example, one may prepare a population of cells to overexpress OB receptor (such as one identified in the Sequence ID's or otherwise denoted herein), or to express a desired form of OB receptor, such as one which is particularly sensitive to OB protein. One may then implant such cells into an individual to increase that individual's sensitivity to OB protein. Such cells may, for example, be liver cells, or bone marrow cells. Alternatively, one may wish to use overexpressing circulating cells such as blood progenitor cells or other blood cells. Cells may be in the form of tissue. Such cells may be cultured prior to transplantation or implantation. Such OB receptor overexpression, or expression of particularly sensitive forms of OB receptor may be accomplished by, for example, altering the regulatory mechanism for expression of OB receptor, such as using homologous recombination techniques as described <u>supra</u>. Thus, provided is a population of host cells modified so that expression of endogenous OB receptor DNA is enhanced.

The present invention provides purified and isolated polypeptide products having part or all of the primary structural conformation (i.e., continuous sequence of amino acid residues) and one or more of the biological

properties (e.g., immunological properties and *in vitro* biological activity) and physical properties (e.g., molecular weight) of naturally-occurring mammalian OB receptor including allelic variants thereof. The term "purified and isolated" herein means substantially free of unwanted substances so that the present polypeptides are useful for an intended purpose. For example, one may have a recombinant human OB receptor substantially free of other human proteins or pathological agents. These polypeptides are also characterized by being the a product of mammalian cells, or the product of chemical synthetic procedures or of procaryotic or eucaryotic host expression (e.g., by bacterial, yeast, higher plant, insect and mammalian cells in culture) of exogenous DNA sequences obtained by genomic or cDNA cloning or by gene synthesis. The products of expression in typical yeast (e.g., *Saccharomyces cerevisiae*) or procaryote (e.g., *E. coli*) host cells are free of association with any mammalian proteins. The products of expression in vertebrate (e.g., non-human mammalian (e.g. COS or CHO) and avian) cells are free of association with any human proteins. Depending upon the host employed, and other factors, polypeptides of the invention may be glycosylated with mammalian or other eucaryotic carbohydrates or may be non-glycosylated. One may modify the nucleic acid so that glycosylation sites are included. Polypeptides of the invention may also include an initial methionine amino acid residue (at position -1 with respect to the first amino acid residue of the mature polypeptide).

In addition to naturally-occurring allelic forms of OB receptor, the present invention also embraces other OB receptor products such as polypeptide analogs of OB receptor and fragments of OB receptor. Following the procedures of the above noted published application by Alton et al. (WO 83/04053), one can readily design and manufacture genes coding for microbial expression of polypeptides having

primary conformations which differ from that herein specified for in terms of the identity or location of one or more residues (e.g., substitutions, terminal and intermediate additions and deletions). Alternately, modifications of cDNA and genomic genes may be readily accomplished by well-known site-directed mutagenesis techniques and employed to generate analogs and derivatives of OB receptor. Such products would share at least one of the biological properties of mammalian OB receptor but may differ in others. As examples, projected products of the invention include those which are foreshortened by e.g., deletions; or those which are more stable to hydrolysis (and, therefore, may have more pronounced or longer lasting effects than naturally-occurring); or which have been altered to delete one or more potential sites for glycosylation (which may result in higher activities for yeast-produced products); or which have one or more cysteine residues deleted or replaced by, e.g., alanine or serine residues and are potentially more easily isolated in active form from microbial systems; or which have one or more tyrosine residues replaced by phenylalanine and bind more or less readily to target proteins or to receptors on target cells. Also comprehended are polypeptide fragments duplicating only a part of the continuous amino acid sequence or secondary conformations within OB receptor, which fragments may possess one activity of mammalian OB receptor (e.g., immunological activity) and not others (e.g., OB protein binding activity).

Of applicability to OB receptor fragments and polypeptide analogs of the invention are reports of the immunological activity of synthetic peptides which substantially duplicate the amino acid sequence extant in naturally-occurring proteins, glycoproteins and nucleoproteins. More specifically, relatively low molecular weight polypeptides have been shown to participate in immune reactions which are similar in duration and extent to the

immune reactions of physiologically significant pr teins such as viral antigens, polypeptide hormones, and the like. Included among the immune reactions of such polypeptides is the provocation of the formation of specific antibodies in immunologically active animals. _See, e.g._, Lerner et al., Cell _23_: 309-310 (1981); Ross et al., Nature _294_: 654-656 (1981); Walter et al., PNAS-USA _77_: 5197-5200 (1980); Lerner et al., PNAS-USA, _78_: 3403-3407 (1981); Walter et al., PNAS-USA _78_: 4882-4886 (1981); Wong et al., PNAS-USA _79_: 5322-5326 (1982); Baron et al., Cell _28_: 395-404 (1982); Dressman et al., Nature _295_: 185-160 (1982); and Lerner, Scientific American _248_: 66-74 (1983). _See, also_, Kaiser et al. Science _223_: 249-255 (1984) relating to biological and immunological activities of synthetic peptides which approximately share secondary structures of peptide hormones but may not share their primary structural conformation.

Thus, the present class of OB receptor proteins includes those having amino acids (according to Seq. ID No. 1):

(a) 1-896;
(b) 22-896;
(c) 23-896;
(d) 29-896
(e) 1-839;
(f) 22-839;
(g) 29-839;
(h) 1-841;
(i) 22-841;
(j) 23-841;
(k) 29-841;
(l) 1-891;
(m) 22-981;
(n) 23-891;
(o) 29-891;

(p) the amino acids of subparts (l) through (o) having the C-terminal amino acids of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5);

(q) amino acids of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

In addition, since the C-terminus region of the above polypeptides diverges at position 892 (with respect to Seq. ID Nos. 1, 3 and 5) one may desire to prepare only the polypeptides which are divergent:

(a) those having only amino acids 892-896 of Seq. ID No. 1;

(b) those having only amino acids 892-933 of Seq. ID No. 3;

(c) those having only amino acids 892-959 of Seq. ID No. 5.

One may modify the OB receptor to create a fusion molecule with other peptide sequence. For example, if one desired to "tag" the OB receptor with an immunogenic peptide, one could construct a DNA which would result in such fusion protein. The tag may be at the N-terminus. Alternatively, since it is apparent that the C-terminus is not necessary for ligand binding activity, one may chemically modify the C-terminus. One may desire, for example, a preparation whereby one or more polymer molecules such as polyethylene glycol molecules are attached. Thus, another aspect of the present invention is chemically modified OB receptor protein.

The present invention also includes that class of polypeptides coded for by portions of the DNA complementary to the protein-coding strand of the human cDNA or genomic DNA sequences of OB receptor i.e., "complementary inverted proteins" as described by Tramontano et al. Nucleic Acid Res. 12: 5049-5059 (1984). Polypeptides or analogs thereof may also contain one or more amino acid analogs, such as peptidomimetics.

One may prepare soluble receptor by elimination of the transmembrane and intracellular regions.

Also comprehended by the invention are pharmaceutical compositions comprising effective amounts of polypeptide products of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in OB receptor therapy. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); covalent attachment of polymers such as polyethylene glycol to the protein (as discussed _supra_, _see_, for example U.S. patent 4,179,337 hereby incorporated by reference); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Such compositions will influence the physical state, stability, rate of _in vivo_ release, and rate of _in vivo_ clearance of OB receptor. _See_, _e.g._, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712 which are herein incorporated by reference.

Generally, an effective amount of the present OB receptor polypeptides will be determined by the age, weight and condition or severity of disease of the recipient. _See_, Remingtons Pharmaceutical Sciences, _supra_, at pages 697-773, herein incorporated by reference. Typically, a dosage of between about 0.001mg/kg body weight/day to about 1g/kg body weight/day, may be used, but more or less, as a skilled practitioner will recognize, may be used. For local (i.e., non-systemic) applications, such as topical applications, the dosing may be between about 0.001g/cm^2 to about 1g/cm^2. Dosing may be one or more times daily, or less frequently, and may be in conjunction with other compositions as

described herein. It should be noted that the present invention is not limited to the dosages recited herein.

Polypeptide products of the invention may be "labeled" by association with a detectable marker substance (e.g., radiolabeled with ^{125}I) to provide reagents useful in detection and quantification of OB receptor in solid tissue and fluid samples such as blood or urine. Nucleic acid products of the invention may also be labeled with detectable markers (such as radiolabels and non-isotopic labels such as biotin) and employed in hybridization processes to locate the human OB receptor gene position and/or the position of any related gene family in a chromosomal map. Nucleic acid sequences which selectively bind the human OB receptor gene are useful for this purpose. They may also be used for identifying human OB receptor gene disorders at the DNA level and used as gene markers for identifying neighboring genes and their disorders. Contemplated herein are kits containing such labelled materials.

The nucleic acids provided herein may also be embodied as part of a kit or article of manufacture. Contemplated is an article of manufacture comprising a packaging material and one or more preparations of the presently provided nucleic acids. Such packaging material will comprise a label indicating that the nucleic acid preparation is useful for detecting OB receptor or OB receptor defects in a biological sample. As such, the kit may optionally include materials to carry out such testing, such as reagents useful for performing DNA or RNA hybridization analysis, or PCR analysis on blood, urine, or tissue samples.

A further embodiment of the invention is selective binding molecules, such as monoclonal antibodies selectively binding OB receptor. The hybridoma technique described originally by Kohler and Milstein Eur. J. Immunol. 6, 511-519 (1976) has been widely applied to produce hybrid cell lines

that secrete high levels of monoclonal antibodies against many specific antigens. Recombinant antibodies, (see Huse et al., Science 246: 1275 (1989)) may also be prepared. Such recombinant antibodies may be further modified, such as by modification of complementarity determining regions to increase or alter affinity, or "humanizing" such antibodies. Such antibodies may be incorporated into a kit for diagnostic purposes, for example. A diagnostic kit may be employed to determine the location and/or amount or OB receptor of an individual. Diagnostic kits may also be used to determine if an individual has receptors which bind OB protein, or those which, to varying degrees, have reduced binding capacity or ability. As stated infra, such antibodies may be prepared using immunogenic portions of an OB receptor protein. Such selective binding molecules may themselves be alternatives to OB protein, and may be formulated for pharmaceutical composition.

RELATED METHODS

The present compositions may be used in therapeutic as well as diagnostic methods.

The present OB receptor proteins or nucleic acids may be used for methods of treatment, or for methods of manufacturing medicaments for treatment. Such treatment includes conditions characterized by excessive production of OB protein, wherein the present OB receptors, particularly in soluble form, may be used to comples to and therefore inactivate such excessive OB protein. This treatment may be accomplished by preparing soluble receptor (e.g., use of the extracellular domain) or by preparation of a population of cells containing such OB receptor, and transplanting such cells into the individual in need thereof. The present OB receptors may also be used for treatment of those having defective OB receptors. For example, one may treat an

individual having defective OB receptors by preparation of a population of cels containing such non-defective OB receptor, and transplanting such cells into an individual. Or, an individual may have an inadequate number of OB receptors, and cells containing such receptors may be transplanted in order to increase the number of OB receptors available to an individual. Such treatment may be for the purpose of modulating weight loss, for therapeutic purposes or solely for cosmetic purposes.

The present OB receptor protein or nucleic acids may be used for diagnostic purposes. For instance, RNAs or DNAs may be used to characterize or detect defects in an individual's OB receptors. For example, an obese individual may possess OB receptors which are characterized by a reduced ability to bind OB protein. The present DNAs may be used to hybridize with the nucleic acid from an individual to detect such defects, such as via PCR techniques. OB receptor protein may be used to characterize an individual's OB protein for its ability to bind to OB receptor, or for other biological activity. For example, one may prepare an assay for the ability of OB protein to alter lipid metabolism by preparing a population of lipid containing cells expressing the OB receptor, and contacting OB protein with such population of cells. Modulation of lipid content, characteristics of lipid or other characteristics may be monitored. For diagnostic purposes, the present protein or nucleic acids may be associated with a detectable labels substance such as a radioactive isotope, a fluorescent or chemiluminescent chemical, or other label available to one skilled in the art. Such nucleic acids may be used for tissue distribution assays (for example, as provided in the working example below) or for other assays to determine the location of OB receptor.

The present OB receptor protein family may be used in methods to obtain OB protein analogs, mimetics or small molecules. One would simply prepare a desired OB receptor

protein, particularly one with capability of binding to native OB protein, and assay the test molecule, which may be labelled with a detectable label substance, for ability to bind to such receptor. Other parameters, such as affinity, and location of binding, may also be ascertained by methods available to those skilled in the art. For example, one could use portions of the present OB receptors, particularly portions in the extracellular domain which are necessary for ligand binding, to determine the location of such binding. One could prepare OB receptors which have various truncations or deletions of regions of the extracellular domain which could be used to determine the location of test molecule binding. One could use an OB receptor known to be defective in native OB binding, such as potentially one from an individual having such defective receptors, and use this as the basis for ascertaining OB protein which would be effective to result in desired biological activity (i.e., weight loss, reduction in blood dyslipidemias or lowering of cholesterol levels, reductin in incidence of diabetes). Other uses include solely cosmetic uses for alteration of body appearance, particularly the removal of fat.

The present OB receptor protein or nucleic ac_is may also be useful to identify substances which "up-regulate" OB protein or receptor. For instance, the temporal expression of OB receptor *in vivo* may abe useful to determine if an administered substance causes an increase or decrease in OB receptor. One may conclude that an increase in OB receptor expression results in modultion of weight or lipid metabolism.

The divergence in the C-terminus may represent OB receptors with different signal transduction abilities. Therefore the different receptor family members may be used for differing assays, depending on the type of signal transduction observed.

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

EXAMPLE 1: IDENTIFICATION OF HUMAN OB RECEPTOR PROTEIN

Human OB receptor protein DNA was identified in a human liver cDNA library in two steps. The first step used two primers in polymerase chain reaction (PCR) primers to amplify a selected 300 base pair region from the human liver cDNA library. The second step used the PCR fragment as a probe to screen the human liver cDNA library. Thirteen clones were obtained, but these were incomplete at the 5' end. A procedure was performed to complete the 5' end to make complete clones. Twelve clones were sequenced. These twelve clones were identified as either "A", "B" or "C" as denoted by the C'terminus of the predicted amino acid sequence.

Polymerase Chain Reaction. The original PCR primer was based on the 5' end and the 3' end of a 416 base pair sequence having GenBank Database Accession No. T73849. This sequence was selected on the basis of a known motif present in cytokine receptors, "WSXWS".

The 5' primer had the sequence 73-96 of the 416 bp sequence. The 3' primer had the sequence 337-360 of the 416 bp sequence.

These primers were used to probe a human cDNA liver library (Stratagene). Standard methods were used.

This resulted in a PCR fragment having the sequence 73-360 of the 416 bp fragment.

Hybridization. The 300 bp PCR fragment was used to probe a human liver cDNA library (Stratagene) using standard

methods. This second hybridization resulted in 13 positive clones. These were partial clones, incomplete at the 5' end.

Completion of the 5' end. Rapid Amplification of cDNA End ("RACE", kit, GIBCO/BRL) was used to obtain the full length clones.

Sequencing results. Sequencing revealed the three types of OB receptor DNAs. Of the thirteen clones, 4 clones were the "A" type (Seq. ID Nos. 1 and 2); 1 clone was the "B" type (Seq. ID Nos. 3 and 4) and 4 clones were of the "C" type (Seq. ID Nos. 5 and 6).

As can be seen from the Sequence Identifications (below), OB receptor A is 896 amino acids long, "B" is 933 amino acids long, and "C" is 959 amino acids long. These different OB receptors are identical at amino acid positions 1-891, and diverge almost completely beginning at position 892. The leader sequence is postulated to be, by hydrophobicity analysis, amino acids 1-21(M-A), 1-22(M-F) or 1-28(M-I), with the mature protein beginning at positions 22(F), 23(N) or 29(T). Based on hydrophobicity analysis, the leader sequence is most likely to be at positions 1-21(M through A). The transmembrane region is likely to begin at either position 840 (A) or 842(L) through position 862(I), 863(S) or 864(M). For OB receptor type A, the last amino acid is located at position 896 and is a lysine (L).

EXAMPLE 2: TISSUE DISTRIBUTION

Tissue distribution was ascertained using two methods. The first method involved using the entire type "A" OB receptor. The second method involved using probes which are specific to the C-terminal region of the protein. Since these C terminal regions are divergent, the second method

detected the tissue distribution of the different members of the OB receptor family.

The first method used a Northern Blot kit (Clontech), using the entire type A OB receptor DNA as a probe. The second method used PCR with primers specific to the nucleic acids encoding the divergent C terminus of the three types. Standard methods were used.

Table 1 shows the results for the Northern Blot and the PCR methods. The "+" indicates the investigator's subjective determination of the strength of signal. For the Northern Blot analysis, a triple "+++" indicates that a result (a dark "band" on the X-ray film) was seen upon overnight exposure of the film. A double "++" indicates that bands were seen at two weeks of exposure. A single "+" indicates that the bands were seen after three weeks of exposure. In addition, using this method, two molecular weights were observed, one at 4 Kb and one at 6.2 Kb. Although distribution was ubiquitous, the strongest signals were seen for ovary, heart and liver. For the PCR analysis, OB receptor "A" was seen in all tissue types tested (prostate, ovary, small intestine, heart, lung, liver and skeletal muscle), type "B" was seen only in lung and liver, and type "C" was seen in ovary, heart, lung and liver.

Table 1

Tissue Distribution of the Novel OB Receptor

	Northern Blot			PCR		
	4 Kb	6.2 Kb		A	B	C
Spleen	-	+				
Thymus	-	+				
Prostate	-	+		+	-	-
Testis	-	+				
Ovary	-	+++		+	-	+
Small Intestine	-	++		+	-	-
Colon	-	-				
Peripheral blood Leukocyte	-	-				
Heart	-	+++		+	-	+
Brain	-	-				
Placenta	-	+				
Lung	+	++		+	+	+
Liver	+++	+++		+	+	+
Skeletal Muscle	-	++		+	-	-
Kidney	-	++				
Pancreas	-	+				

EXAMPLE 3: IDENTIFICATION OF HUMAN OB RECEPTOR GENOMIC DNA AND CHROMOSOME LOCALIZATION

The full length human OB receptor genomic DNA was also prepared. OB receptor "A" cDNA, in its entirety, was used as a probe against a human genomic DNA library, using materials and methods from a commercially available kit (Genome Systems, using a human genomic library in a P1 vector). A single positive clone was detected. There are introns located at (with respect to OB receptor "A" DNA) base pair number: 559, 1059, 1350, 1667, 1817, 1937, 2060, 2277, 2460, 2662, and 2738.

The human OB receptor gene was localized to human chromosome 1P31 by FISH analysis (Genome Systems). Human chromosome 1 is thought to correspond to mouse chromosome 4C7, which is presumed to be the location of the *db* locus.

EXAMPLE 4: PREPARATION OF EXPRESSION VECTORS

Recombinant human OB receptor expression vectors have been prepared for expression in mammalian cells. As indicated above, expression may also be in non-mammalian cells, such as bacterial cells. The type "A" cDNA (Seq. ID No. 2) was placed into a commercially available mammalian vector (CEP4, Invitrogen) for expression in mammalian cells, including the commercially available human embryonic kidney cell line, "293". For expression in bacterial cells, one would typically eliminate that portion encoding the leader sequence (e.g., potentially amino acids 1-21, 1-22 or 1-28). One may add an additional methionyl at the N-terminus for bacterial expression. Additionally, one may substitute the native leader sequence with a different leader sequence, or other sequence for cleavage for ease of expression.

EXAMPLE 5: PREPARATION OF SELECTIVE BINDING MOLECULES

Animals were immunized for the preparation of polyclonal antibodies using the following peptides (with respect to the numbering of the amino acids for OB receptor A, Seq. ID No. 1): 54-64; 91-100; 310-325; 397-406; 482-496; 874-885; and, with respect to amino acids of OB receptor "C" (Seq. ID No. 5), 910-929.

Other immunogenic peptides may be used. Polyclonal, monospecific polyclonal, monoclonal, antibody fragments, and recombinant antibodies may be prepared using methods available to those skilled in the art.

One may further use recombinant techniques or peptide synthesis methods to alter the character of the such selective binding molecules. This may be accomplished by preparing recombinant antibodies having altered complementarity determining regions (sometimes referred to in the art as "CDR's") to, for example "humanize" the antibodies by using human F_c (constant) regions. Other types of recombinant antibodies, for example, those having CDR's altered to enhance affinity or selectivity to one or more members of the OB receptor family, may be prepared and used using methods available to those skilled in the art. See Winter et al., Nature 349: 293-299 (1991).

The present OB receptor protein may be used as an assay to screen for desired selective binding molecules. Such assay may be based on binding capability, or biological activity, or, other means of detecting signal transduction. For example, if one were to prepare a series of modified antibodies, one could test them for affinity (i.e, binding strength) against the target OB receptor.

The selective binding molecules may be useful for diagnostic purposes, such as tissue distribution analysis, or to diagnose the relative affinity of an individual's OB receptors for such selective binding molecule to determine the functionality of an individual's OB receptor during a course of therapy. Selective binding molecules may be alternative therapeutic or cosmetic products to OB protein.

While the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

Human OB Receptor "A" Amino Acid Sequence (Seq. ID No. 1(Amino Acid, single letter abbreviation, "*" indicating stop codon):

1	MICQKFCVVL	LHWEFIYVIT	AFNLSYPITP	WRFKLSCMPP	NSTYDYFLLP
51	AGLSKNTSNS	NGHYETAVEP	KFNSSGTHFS	NLSKTTFHCC	FRSEQDRNCS
101	LCADNIEGKT	FVSTVNSLVF	QQIDANWNIQ	CWLKGDLKLF	ICYVESLFKN
151	LFRNYNYKVH	LLYVLPEVLE	DSPLVPQKGS	FQMVHCNCSV	HECCECLVPV
201	PTAKLNDTLL	MCLKITSGGV	IFQSPLMSVQ	PINMVKPDPP	LGLHMEITDD
251	GNLKISWSSP	PLVPFPLQYQ	VKYSENSTTV	IREADKIVSA	TSLLVDSILP
301	GSSYEVQVRG	KRLDGPGIWS	DWSTPRVFTT	QDVIYFPPKI	LTSVGSNVSF
351	HCIYKKENKI	VPSKEIVWWM	NLAEKIPQSQ	YDVVSDHVSK	VTFFNLNETK
401	PRGKFTYDAV	YCCNEHECHH	RYAELYVIDV	NINISCETDG	YLTKMTCRWS
451	TSTIQSLAES	TLQLRYHRSS	LYCSDIPSIH	PISEPKDCYL	QSDGFYECIF
501	QPIFLLSGYT	MWIRINHSLG	SLDSPPTCVL	PDSVVKPLPP	SSVKAEITIN
551	IGLLKISWEK	PVFPENNLQF	QIRYGLSGKE	VQWKMYEVYD	AKSKSVSLPV
601	PDLCAVYAVQ	VRCKRLDGLG	YWSNWSNPAY	TVVMDIKVPM	RGPEFWRIIN
651	GDTMKKEKNV	TLLWKPLMKN	DSLCSVQRYV	INHHTSCNGT	WSEDVGNHTK
701	FTFLWTEQAH	TVTVLAINSI	GASVANFNLT	FSWPMSKVNI	VQSLSAYPLN
751	SSCVIVSWIL	SPSDYKLMYF	IIEWKNLNED	GEIKWLRISS	SVKKYYIHDH
801	FIPIEKYQFS	LYPIFMEGVG	KPKIINSFTQ	DDIEKHQSDA	GLYVIVPVII
851	SSSILLLGTL	LISHQRMKKL	FWEDVPNPKN	CSWAQGLNFQ	KRTDIL*SLI
901	MITTDEPNVP	TSQQSIEY*K	IFTF*RRGAN	LKKIQLNF*E	LTYGGLC*FR
951	T*NRCVNLGS	KCRFESSLDV	*L		

Human OB Receptor "A" DNA Sequence (Seq. ID No. 2 (DNA)):

```
1     CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA
51    CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTTACA
101   TTGGGAATTT ATTTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATTA
151   CTCCTTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATTC AACCTATGAC
201   TACTTCCTTT TGCCTGCTGG ACTCTCAAAG AATACTTCAA ATTCGAATGG
251   ACATTATGAG ACAGCTGTTG AACCTAAGTT TAATTCAAGT GGTACTCACT
301   TTTCTAACTT ATCCAAAACA ACTTTCCACT GTTGCTTTCG GAGTGAGCAA
351   GATAGAAACT GCTCCTTATG TGCAGACAAC ATTGAAGGAA AGACATTTGT
401   TTCAACAGTA AATTCTTTAG TTTTTCAACA AATAGATGCA AACTGGAACA
451   TACAGTGCTG GCTAAAAGGA GACTTAAAAT TATTCATCTG TTATGTGGAG
501   TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTTT
551   ATATGTTCTG CCTGAAGTGT TAGAAGATTC ACCTCTGGTT CCCCAAAAAG
601   GCAGTTTTCA GATGGTTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA
651   TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG
701   TTTGAAAATC ACATCTGGTG GAGTAATTTT CCAGTCACCT CTAATGTCAG
751   TTCAGCCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG
801   GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT
851   GGTACCATTT CCACTTCAAT ATCAAGTGAA ATATTCAGAG AATTCTACAA
901   CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA
951   GACAGTATAC TTCCTGGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG
1001  ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA
1051  CCACACAAGA TGTCATATAC TTTCCACCTA AAATTCTGAC AAGTGTTGGG
1101  TCTAATGTTT CTTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC
1151  CTCAAAAGAG ATTGTTTGGT GGATGAATTT AGCTGAGAAA ATTCCTCAAA
1201  GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTTCAAT
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1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG

1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATTA TATGTGATTG

1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG

1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT

1451 GCAATTGAGG TATCATAGGA GCAGCCTTTA CTGTTCTGAT ATTCCATCTA

1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTTT

1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAATGTG

1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG

1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA

1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT

1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA

1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAAA ATCAAAATCT

1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG

1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG

1951 CCTACACAGT TGTCATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT

2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACTTT

2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT

2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG

2151 GGAAATCACA CGAAATTCAC TTTCCTGTGG ACAGAGCAAG CACATACTGT

2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTTGCA AATTTTAATT

2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT

2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTTCCTGGA TACTATCACC

2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG

2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT

2451 TATATCCATG ATCATTTTAT CCCCATTGAG AAGTACCAGT TCAGTCTTTA

2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTTCA

2551 CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

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2601  GTGCCAGTAA TTATTTCCTC TTCCATCTTA TTGCTTGGAA CATTATTAAT
2651  ATCACACCAA AGAATGAAAA AGCTATTTTG GGAAGATGTT CCGAACCCCA
2701  AGAATTGTTC CTGGGCACAA GGACTTAATT TTCAGAAGAG AACGGACATT
2751  CTTTGAAGTC TAATCATGAT CACTACAGAT GAACCCAATG TGCCAACTTC
2801  CCAACAGTCT ATAGAGTATT AGAAGATTTT TACATTTTGA AGAAGGGGAG
2851  CAAATCTAAA AAAAATTCAG TTGAACTTCT GAGAGTTAAC ATATGGTGGA
2901  TTATGTTGAT TTAGAACTTA AAATAGATGT GTAAATTTGG GTTCAAAATG
2951  TAGATTTGAG TCCAGTTTGG ATGTGTGATT AATTTTCAAA TCATCTAAAG
3001  TTTAAAAGTA GTATTCATGA TTTCTGGCTT TTGATTTGCC ATATTCCTGG
3051  TCATAAAACA TTAAGAAAAT TATGGCTGTT GCTGTCATTA CATATCTATT
3101  AAATGTCATC AAATATGTAG TAGACAATTT TGTAATTAGG TGAACTCTAA
3151  AACTGCAACA TCTGACAAAT TGCTTTAAAA ATACAATGAT TAT
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Human OB Receptor "B" Amino Acid Sequence (Seq. ID No. 3 (Amino Acid)):

```
1    MICQKFCVVL LHWEFIYVIT AFNLSYPITP WRFKLSCMPP NSTYDYFLLP
51   AGLSKNTSNS NGHYETAVEP KFNSSGTHFS NLSKTTFHCC FRSEQDRNCS
101  LCADNIEGKT FVSTVNSLVF QQIDANWNIQ CWLKGDLKLF ICYVESLFKN
151  LFRNYNYKVH LLYVLPEVLE DSPLVPQKGS FQMVHCNCSV HECCECLVPV
201  PTAKLNDTLL MCLKITSGGV IFQSPLMSVQ PINMVKPDPP LGLHMEITDD
251  GNLKISWSSP PLVPFPLQYQ VKYSENSTTV IREADKIVSA TSLLVDSILP
301  GSSYEVQVRG KRLDGPGIWS DWSTPRVFTT QDVIYFPPKI LTSVGSNVSF
351  HCIYKKENKI VPSKEIVWWM NLAEKIPQSQ YDVVSDHVSK VTFFNLNETK
401  PRGKFTYDAV YCCNEHECHH RYAELYVIDV NINISCETDG YLTKMTCRWS
451  TSTIQSLAES TLQLRYHRSS LYCSDIPSIH PISEPKDCYL QSDGFYECIF
501  QPIFLLSGYT MWIRINHSLG SLDSPPTCVL PDSVVKPLPP SSVKAEITIN
551  IGLLKISWEK PVFPENNLQF QIRYGLSGKE VQWKMYEVYD AKSKSVSLPV
601  PDLCAVYAVQ VRCKRLDGLG YWSNWSNPAY TVVMDIKVPM RGPEFWRIIN
651  GDTMKKEKNV TLLWKPLMKN DSLCSVQRYV INHHTSCNGT WSEDVGNHTK
701  FTFLWTEQAH TVTVLAINSI GASVANFNLT FSWPMSKVNI VQSLSAYPLN
751  SSCVIVSWIL SPSDYKLMYF IIEWKNLNED GEIKWLRISS SVKKYYIHDH
801  FIPIEKYQFS LYPIFMEGVG KPKIINSFTQ DDIEKHQSDA GLYVIVPVII
851  SSSILLLGTL LISHQRMKKL FWEDVPNPKN CSWAQGLNFQ KKRLSIFLSS
901  IQHQ*HVVLF FWSLKQFQKI SVLIHHGKIK MR*CQQLWSL YFQQQILKRV
951  LFVLVTSSTV LTSLRLRVLR *PMRTKARDN PLLNTPR*SA TLNQVKLVK
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Human OB Receptor "B" DNA Sequence (Seq. ID No. 4 (DNA)):

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1     CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA
51    CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTTACA
101   TTGGGAATTT ATTTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATTA
151   CTCCTTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATTC AACCTATGAC
201   TACTTCCTTT TGCCTGCTGG ACTCTCAAAG AATACTTCAA ATTCGAATGG
251   ACATTATGAG ACAGCTGTTG AACCTAAGTT TAATTCAAGT GGTACTCACT
301   TTTCTAACTT ATCCAAAACA ACTTTCCACT GTTGCTTTCG GAGTGAGCAA
351   GATAGAAACT GCTCCTTATG TGCAGACAAC ATTGAAGGAA AGACATTTGT
401   TTCAACAGTA AATTCTTTAG TTTTTCAACA AATAGATGCA AACTGGAACA
451   TACAGTGCTG GCTAAAAGGA GACTTAAAAT TATTCATCTG TTATGTGGAG
501   TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTTT
551   ATATGTTCTG CCTGAAGTGT TAGAAGATTC ACCTCTGGTT CCCCAAAAAG
601   GCAGTTTTCA GATGGTTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA
651   TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG
701   TTTGAAAATC ACATCTGGTG GAGTAATTTT CCAGTCACCT CTAATGTCAG
751   TTCAGCCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG
801   GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT
851   GGTACCATTT CCACTTCAAT ATCAAGTGAA ATATTCAGAG AATTCTACAA
901   CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA
951   GACAGTATAC TTCCTGGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG
1001  ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA
1051  CCACACAAGA TGTCATATAC TTTCCACCTA AAATTCTGAC AAGTGTTGGG
1101  TCTAATGTTT CTTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC
1151  CTCAAAAGAG ATTGTTTGGT GGATGAATTT AGCTGAGAAA ATTCCTCAAA
1201  GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTTCAAT
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1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG
1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATTA TATGTGATTG
1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG
1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT
1451 GCAATTGAGG TATCATAGGA GCAGCCTTTA CTGTTCTGAT ATTCCATCTA
1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTTT
1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAATGTG
1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG
1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA
1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT
1751 CTTTCCAGAG AATAACCTTC AATTCCAG[illegible]T TCGCTATGGT TTAAGTGGAA
1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAAA ATCAAAATCT
1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG
1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG
1951 CCTACACAGT TGTCATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT
2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACTTT
2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT
2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG
2151 GGAAATCACA CGAAATTCAC TTTCCTGTGG ACAGAGCAAG CACATACTGT
2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTTGCA AATTTTAATT
2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT
2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTTCCTGGA TACTATCACC
2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG
2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT
2451 TATATCCATG ATCATTTTAT CCCCATTGAG AAGTACCAGT TCAGTCTTTA
2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTTCA
2551 CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT

2601 GTGCCAGTAA TTATTTCCTC TTCCATCTTA TTGCTTGGAA CATTATTAAT
2651 ATCACACCAA AGAATGAAAA AGCTATTTTG GGAAGATGTT CCGAACCCCA
2701 AGAATTGTTC CTGGGCACAA GGACTTAATT TTCAGAAGAA ACGTTTGAGC
2751 ATCTTTTTAT CAAGCATACA GCATCAGTGA CATGTGGTCC TCTTCTTTTG
2801 GAGCCTGAAA CAATTTCAGA AGATATCAGT GTTGATACAT CATGGAAAAA
2851 TAAAGATGAG ATGATGCCAA CAACTGTGGT CTCTCTACTT TCAACAACAG
2901 ATCTTGAAAA GGGTTCTGTT TGTTTTAGTG ACCAGTTCAA CAGTGTTAAC
2951 TTCTCTGAGG CTGAGGGTAC TGAGGTAACC TATGAGGACG AAAGCCAGAG
3001 ACAACCCTTT GTTAAATACG CCACGCTGAT CAGCAACTCT AAACCAAGTG
3051 AAACTGGTGA AGA

Human OB Receptor "C" Amino Acid Sequence (Seq. ID No. 5 (Amino Acid)):

1	MICQKFCVVL	LHWEFIYVIT	AFNLSYPITP	WRFKLSCMPP	NSTYDYFLLP
51	AGLSKNTSNS	NGHYETAVEP	KFNSSGTHFS	NLSKTTFHCC	FRSEQDRNCS
101	LCADNIEGKT	FVSTVNSLVF	QQIDANWNIQ	CWLKGDLKLF	ICYVESLFKN
151	LFRNYNYKVH	LLYVLPEVLE	DSPLVPQKGS	FQMVHCNCSV	HECCECLVPV
201	PTAKLNDTLL	MCLKITSGGV	IFQSPLMSVQ	PINMVKPDPP	LGLHMEITDD
251	GNLKISWSSP	PLVPFPLQYQ	VKYSENSTTV	IREADKIVSA	TSLLVDSILP
301	GSSYEVQVRG	KRLDGPGIWS	DWSTPRVFTT	QDVIYFPPKI	LTSVGSNVSF
351	HCIYKKENKI	VPSKEIVWWM	NLAEKIPQSQ	YDVVSDHVSK	VTFFNLNETK
401	PRGKFTYDAV	YCCNEHECHH	RYAELYVIDV	NINISCETDG	YLTKMTCRWS
451	TSTIQSLAES	TLQLRYHRSS	LYCSDIPSIH	PISEPKDCYL	QSDGFYECIF
501	QPIFLLSGYT	MWIRINHSLG	SLDSPPTCVL	PDSVVKPLPP	SSVKAEITIN
551	IGLLKISWEK	PVFPENNLQF	QIRYGLSGKE	VQWKMYEVYD	AKSKSVSLPV
601	PDLCAVYAVQ	VRCKRLDGLG	YWSNWSNPAY	TVVMDIKVPM	RGPEFWRIIN
651	GDTMKKEKNV	TLLWKPLMKN	DSLCSVQRYV	INHHTSCNGT	WSEDVGNHTK
701	FTFLWTEQAH	TVTVLAINSI	GASVANFNLT	FSWPMSKVNI	VQSLSAYPLN
751	SSCVIVSWIL	SPSDYKLMYF	IIEWKNLNED	GEIKWLRISS	SVKKYYIHDH
801	FIPIEKYQFS	LYPIFMEGVG	KPKIINSFTQ	DDIEKHQSDA	GLYVIVPVII
851	SSSILLLGTL	LISHQRMKKL	FWEDVPNPKN	CSWAQGLNFQ	KMLEGSMFVK
901	SHHHSLISST	QGHKHCGRPQ	GPLHRKTRDL	CSLVYLLTLP	PLLSYDPAKS
951	PSVRNTQE*S	IKKKKKKLEG			

Human OB Receptor "C" DNA Sequence (Seq. ID No. 6 (DNA)):

```
1     CCGCCGCCAT CTCTGCCTTC GGTCGAGTTG GACCCCCGGA TCAAGGTGTA
51    CTTCTCTGAA GTAAGATGAT TTGTCAAAAA TTCTGTGTGG TTTTGTTACA
101   TTGGGAATTT ATTTATGTGA TAACTGCGTT TAACTTGTCA TATCCAATTA
151   CTCCTTGGAG ATTTAAGTTG TCTTGCATGC CACCAAATTC AACCTATGAC
201   TACTTCCTTT TGCCTGCTGG ACTCTCAAAG AATACTTCAA ATTCGAATGG
251   ACATTATGAG ACAGCTGTTG AACCTAAGTT TAATTCAAGT GGTACTCACT
301   TTTCTAACTT ATCCAAAACA ACTTTCCACT GTTGCTTTCG GAGTGAGCAA
351   GATAGAAACT GCTCCTTATG TGCAGACAAC ATTGAAGGAA AGACATTTGT
401   TTCAACAGTA AATTCTTTAG TTTTTCAACA AATAGATGCA AACTGGAACA
451   TACAGTGCTG GCTAAAAGGA GACTTAAAAT TATTCATCTG TTATGTGGAG
501   TCATTATTTA AGAATCTATT CAGGAATTAT AACTATAAGG TCCATCTTTT
551   ATATGTTCTG CCTGAAGTGT TAGAAGATTC ACCTCTGGTT CCCCAAAAAG
601   GCAGTTTTCA GATGGTTCAC TGCAATTGCA GTGTTCATGA ATGTTGTGAA
651   TGTCTTGTGC CTGTGCCAAC AGCCAAACTC AACGACACTC TCCTTATGTG
701   TTTGAAAATC ACATCTGGTG GAGTAATTTT CCAGTCACCT CTAATGTCAG
751   TTCAGCCCAT AAATATGGTG AAGCCTGATC CACCATTAGG TTTGCATATG
801   GAAATCACAG ATGATGGTAA TTTAAAGATT TCTTGGTCCA GCCCACCATT
851   GGTACCATTT CCACTTCAAT ATCAAGTGAA ATATTCAGAG AATTCTACAA
901   CAGTTATCAG AGAAGCTGAC AAGATTGTCT CAGCTACATC CCTGCTAGTA
951   GACAGTATAC TTCCTGGGTC TTCGTATGAG GTTCAGGTGA GGGGCAAGAG
1001  ACTGGATGGC CCAGGAATCT GGAGTGACTG GAGTACTCCT CGTGTCTTTA
1051  CCACACAAGA TGTCATATAC TTTCCACCTA AAATTCTGAC AAGTGTTGGG
1101  TCTAATGTTT CTTTTCACTG CATCTATAAG AAGGAAAACA AGATTGTTCC
1151  CTCAAAAGAG ATTGTTTGGT GGATGAATTT AGCTGAGAAA ATTCCTCAAA
1201  GCCAGTATGA TGTTGTGAGT GATCATGTTA GCAAAGTTAC TTTTTTCAAT
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1251 CTGAATGAAA CCAAACCTCG AGGAAAGTTT ACCTATGATG CAGTGTACTG

1301 CTGCAATGAA CATGAATGCC ATCATCGCTA TGCTGAATTA TATGTGATTG

1351 ATGTCAATAT CAATATCTCA TGTGAAACTG ATGGGTACTT AACTAAAATG

1401 ACTTGCAGAT GGTCAACCAG TACAATCCAG TCACTTGCGG AAAGCACTTT

1451 GCAATTGAGG TATCATAGGA GCAGCCTTTA CTGTTCTGAT ATTCCATCTA

1501 TTCATCCCAT ATCTGAGCCC AAAGATTGCT ATTTGCAGAG TGATGGTTTT

1551 TATGAATGCA TTTTCCAGCC AATCTTCCTA TTATCTGGCT ACACAATGTG

1601 GATTAGGATC AATCACTCTC TAGGTTCACT TGACTCTCCA CCAACATGTG

1651 TCCTTCCTGA TTCTGTGGTG AAGCCACTGC CTCCATCCAG TGTGAAAGCA

1701 GAAATTACTA TAAACATTGG ATTATTGAAA ATATCTTGGG AAAAGCCAGT

1751 CTTTCCAGAG AATAACCTTC AATTCCAGAT TCGCTATGGT TTAAGTGGAA

1801 AAGAAGTACA ATGGAAGATG TATGAGGTTT ATGATGCAAA ATCAAAATCT

1851 GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG

1901 CTGTAAGAGG CTAGATGGAC TGGGATATTG GAGTAATTGG AGCAATCCAG

1951 CCTACACAGT TGTCATGGAT ATAAAAGTTC CTATGAGAGG ACCTGAATTT

2001 TGGAGAATAA TTAATGGAGA TACTATGAAA AAGGAGAAAA ATGTCACTTT

2051 ACTTTGGAAG CCCCTGATGA AAAATGACTC ATTGTGCAGT GTTCAGAGAT

2101 ATGTGATAAA CCATCATACT TCCTGCAATG GAACATGGTC AGAAGATGTG

2151 GGAAATCACA CGAAATTCAC TTTCCTGTGG ACAGAGCAAG CACATACTGT

2201 TACGGTTCTG GCCATCAATT CAATTGGTGC TTCTGTTGCA AATTTTAATT

2251 TAACCTTTTC ATGGCCTATG AGCAAAGTAA ATATCGTGCA GTCACTCAGT

2301 GCTTATCCTT TAAACAGCAG TTGTGTGATT GTTTCCTGGA TACTATCACC

2351 CAGTGATTAC AAGCTAATGT ATTTTATTAT TGAGTGGAAA AATCTTAATG

2401 AAGATGGTGA AATAAAATGG CTTAGAATCT CTTCATCTGT TAAGAAGTAT

2451 TATATCCATG ATCATTTTAT CCCCATTGAG AAGTACCAGT TCAGTCTTTA

2501 CCCAATATTT ATGGAAGGAG TGGGAAAACC AAAGATAATT AATAGTTTCA

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2551  CTCAAGATGA TATTGAAAAA CACCAGAGTG ATGCAGGTTT ATATGTAATT
2601  GTGCCAGTAA TTATTTCCTC TTCCATCTTA TTGCTTGGAA CATTATTAAT
2651  ATCACACCAA AGAATGAAAA AGCTATTTTG GGAAGATGTT CCGAACCCCA
2701  AGAATTGTTC CTGGGCACAA GGACTTAATT TTCAGAAGAT GCTTGAAGGC
2751  AGCATGTTCG TTAAGAGTCA TCACCACTCC CTAATCTCAA GTACCCAGGG
2801  ACACAAACAC TGCGGAAGGC CACAGGGTCC TCTGCATAGG AAAACCAGAG
2851  ACCTTTGTTC ACTTGTTTAT CTGCTGACCC TCCCTCCACT ATTGTCCTAT
2901  GACCCTGCCA AATCCCCCTC TGTGAGAAAC ACCCAAGAAT GATCAATAAA
2951  AAAAAAAAAA AAAAAACTCG AGGGGG
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CLAIMS

1. An OB receptor protein having part or all of the amino acid sequence according to Seq. ID No. 1.

2. An OB receptor protein selected from among those having amino acids (according to Seq. ID No. 1):

(a) 1-896;
(b) 22-896;
(c) 23-896;
(d) 29-896
(e) 1-839;
(f) 22-839;
(g) 29-839;
(h) 1-841;
(i) 22-841;
(j) 23-841;
(k) 29-841;
(l) 1-891;
(m) 22-981;
(n) 23-891;
(o) 29-891;
(p) of subparts (l) through (o) having the C-terminal amino acids of OB receptor B (Seq. ID No. 3) or C (Seq. ID. No. 5); and
(q) of subparts b, c, d, f, g, i, j, k, m, n, o, and any of (p) lacking a leader sequence, which have an N-terminal methionyl residue.

3. A DNA molecule encoding an OB receptor protein of claim 1 or 2.

4. A DNA molecule encoding an OB receptor protein selected from the group consisting of:

(a) that of Seq. ID. Nos. 2, 4, or 6;
(b) a DNA which hybridizes to a DNA of subpart (a)

(c) a DNA which, but for the degeneracy of the genetic code would hybridize to a DNA of subpart (a) or (b).

5. A biologically functional viral or plasmid vector containing a DNA of claim 3.

6. A biologically functional viral or plasmid vector containing a DNA of claim 4.

7. A prokaryotic or eukaryotic host cell containing the vector of claim 5.

8. A prokaryotic or eukaryotic host cell containing the vector of claim 6.

9. A host cell modified so that expression of endogenous OB receptor DNA is enhanced.

10. A host cell of claim 9 which is an isolated human host cell.

11. A process for producing an OB receptor comprised of culturing, under suitable conditions, a host cell of claim 7 or 8, and obtaining the OB receptor produced.

12. A selective binding molecule which selectively binds an OB receptor.

13. A selective binding molecule of claim 12 which selectively binds the extracellular domain of an OB receptor.

14. An OB receptor polypeptide selected from among:
(a) those having only amino acids 892-896 of Seq. ID No. 1;
(b) those having only amino acids 892-933 of Seq. ID No. 3;
(c) those having only amino acids 892-959 of Seq. ID No. 5.

15. A DNA encoding the OB receptor polypeptide of claim 14.

16. A biologically functional plasmid or viral vector containing the DNA of claim 15.

17. A prokaryotic or eukaryotic host cell containing the vector of claim 16.

18. A process for producing an OB receptor polypeptide comprised of culturing, under suitable conditions, the host cell of claim 17, and otaining the OB receptor polypeptide produced.

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DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or a joint inventor (if plural names are listed below) of the invention entitled

OB PROTEIN RECEPTOR AND RELATED COMPOSITIONS AND METHODS

which is described and claimed in the specification which:

[ ] is attached hereto.
[X] was filed on JANUARY 4, 1996
as Application Serial No.: 08/582825
and was amended on ______________ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

3 Ron K. Levy, Registration No.: 31,539, Steven M. Odre, Registration No.: 29,094, and Karol M. Pessin, Registration No. 34,899, said attorney(s)/agent(s) to have in addition full power of revocation, including the power to revoke any power herein granted.

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Date: March 21, 1996

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Assistant Commissioner of Patents, Washington, D.C. 20231, on the date appearing below.

3/29/96
Date

[signature]
Signature

DECLARATION AND POWER OF ATTORNEY (cont'd)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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